Effect of selected endophytes on *Cicer arietinum* L. under salinity Stress

Synopsis

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INTRODUCTION

According to WHO, 2007 our diet must include sufficient and high-quality dietary protein with essential amino acids to gain optimal growth and health. Presently it is being accomplished by the consumption of meat in most parts of the world. On the other hand, the consumption of meat is becoming a threat to the environment due to the highest greenhouse gas footprints. (Clune et al., 2017; Ritchie and Roser 2020). Legume crops can provide greater care for the environment and sustainability since they are a less expensive source of proteins as compared to those obtained from animals (Iriti et al., 2017; Bourre et al., 2019) and enrich the soil through nitrogen fixation.

Chickpea (*Cicer arietinum* L.) is an important annual legume crop that belongs to the genus *Cicer* (Family: Leguminosae, Fabaceae) and is also recognized as "Garbanzo bean" or "Bengal gram" (Gaur et al., 2010). Chickpea are classified into two types: desi and kabuli. The seed coat of the desi type is thick and coloured (mostly brown), whereas the seed coat of the kabuli type is thin and cream-coloured (Knights and Hobson, 2016). Chickpea seeds have protein concentrations ranging from 16.7% - 30.6% and 12.6% - 29% for desi and kabuli types respectively and are 2-3-fold higher than in cereal grains(8 - 16%). Chickpea proteins have a relatively high content of the essential amino acid lysine (4.9 g/100g - 6.9 g/100g) as compared to cereal grains (2.8 g/100g) (Gangola et al., 2014).

It is extensively grown worldwide, covering over 50 countries of Asia, Africa, Europe, Australia, North America and South America. It is the third-largest food legume produced worldwide (Rasheed et al., 2021; Grasso et al., 2022) and the world's second-most consumed legume crop, cultivated in more than 50 countries worldwide. Globally, India is alone a major producer of chickpea, contributing for 65% of the total chickpea production (Merga and Haji, 2019), but it is highly sensitive to salinity stress which causes yield loss of 8 to 10% globally. Soil salinity is one of the major inevitable problems in arid and semi-arid regions where evapotranspiration exceeds precipitation, affecting about 80 million ha of arable land (Flowers et al., 2010).

Chickpea is a boon for diabetics and is an excellent source of important nutrients such as vitamins A, C, E, K, B1–B3, B5, B6, B9 and minerals (Fe, Zn, Mg and Ca) which have beneficial effects on human health. By 2050, the world population can cross 9 billion, and in order to feed the millions, chickpea production should also be increased. Moreover, it is an important legume that is crucial for food,

nutrition, health security and the livelihood of small-scale farmers with poor resources, especially in developing countries (Koul et al., 2022).

The most significant abiotic stress is salinity (Chen et al., 2009). It is a significant stressor that significantly affects crop productivity. Saline soil conditions have an impact on plant growth by increasing osmotic stress and the toxicity of ions (Bromham, 2015). Salinity adversely affects several morphological features and physiological processes leading to a reduction in growth, a decrease in water status, chlorophyll, ion balance, photosynthesis, nodulation, nitrogen fixation etc., and an increase in reactive oxygen species (ROS), lipid peroxidation thus leading to membrane dysfunction (Flowers et al., 2010; Kumar et al., 2017). The growing demands for crop production are consistently threatened by the escalating salinization of agricultural land (Rozema and Flowers, 2008). However, some plants have effectively adapted and evolved in response to salinity. In arid and semi-arid areas, irrigation is one of the major causes of soil salinization, which is a serious form of soil degradation (Rengasamy et al., 2010).

Around the world, salinity affects more than 900 million hectares of land, reducing the productivity of valuable crops. According to several studies (Flowers and Yeo, 1995; Maser et al., 2002; Munns, 2002; Roy et al., 2014), increased salinity in the soil reduces plant's capacity to absorb water, which can result in water scarcity. It can also cause salt to accumulate in plant parts, which stunts growth by impairing metabolic processes and lowering photosynthetic efficiency. By 2050, it is predicted that salinization, water scarcity, and climate change are expected to have reduced 50% of all arable lands (Wang et al., 2003), which will make it more difficult to meet the world's rising food demand (Cobb et al., 2013). Therefore, breeding stress-tolerant crops, especially those that can withstand salinity and drought, is important for agriculture and necessitates, a thorough understanding of physiological traits and normal fluctuations.

Endophytes an endosymbiotic microorganism, often bacteria or fungi that colonize within a plant for atleast part of its life cycle without causing any visible symptoms. In 1887, De Bary coined the term "Endophyte" (in Greek "*Endon"* = within; "*Phyton"* = plant). They have the ability to invade inside the plant hosts and colonize the intercellular spaces and the xylem vessels (Tan and Zou, 2001).

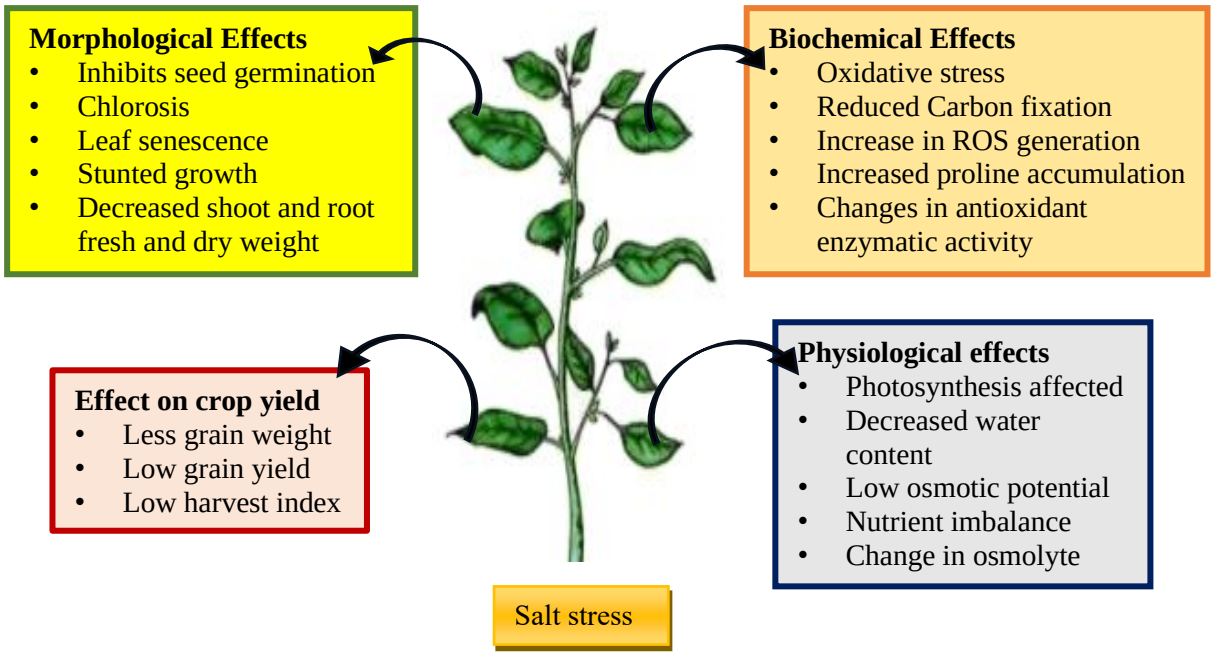

Fig. 1: Effect of salinity stress on plants.

Endophytes exist in a broad range of plant tissues and colonize the plant systemically with microbial colonies. They are ubiquitous and found in almost all species of plants. They live symbiotically within the plants and show no visible symptoms of infection or disease (Bacon and White, 2000). According to most of the studies, there has been a symbiotic relationship between plants and endophytes and it is of benefit for both parties: the endophytic bacteria or fungi gets benefit because of the high nutrient availability inside the plant while the plants benefit through the gain of tolerance against biotic and abiotic stress, promotion of growth, and nutrient accessibility, protection from pathogens (Martinez-Klimova et al., 2016).

By creating certain metabolic compounds, endophytes assist plants in adapting to their environment and promoting plant growth. Plants control the growth of endophytes. In order to protect their host from being infected by bacteria, fungi, insects, and other species, some endophytes can be used as biological control agents in sustainable agricultural production (Zhang, 2019). They achieve this by producing metabolic compounds. Endophytes produce a wide range of chemicals, such as alkaloids, terpenoids, steroids, lignin, quinone, lactones, phenolic compounds, etc. that have positive effects on their host plants, such as promoting plant growth, fixing nitrogen, and supplying resistance to biotic and abiotic stresses like drought, salinity, herbivory, etc. (Schulz B et al., 2002). This boosts the plant's immune

system and protects it against infections.

Endophytes derived from plants growing in coastal saline soils and dry soils demonstrate enhanced production in dry and salty settings, indicating a high commercialization potential (Flowers and Yeo, 1995; Lucero et al., 2008; Yuan et al., 2016). By solubilizing ions and fixing atmospheric nitrogen, endophytes provide nutrients to the plants. This increases plant immunity and shields plants from infection by preventing disease in plants (Barac et al., 2004; Doty et al., 2009).

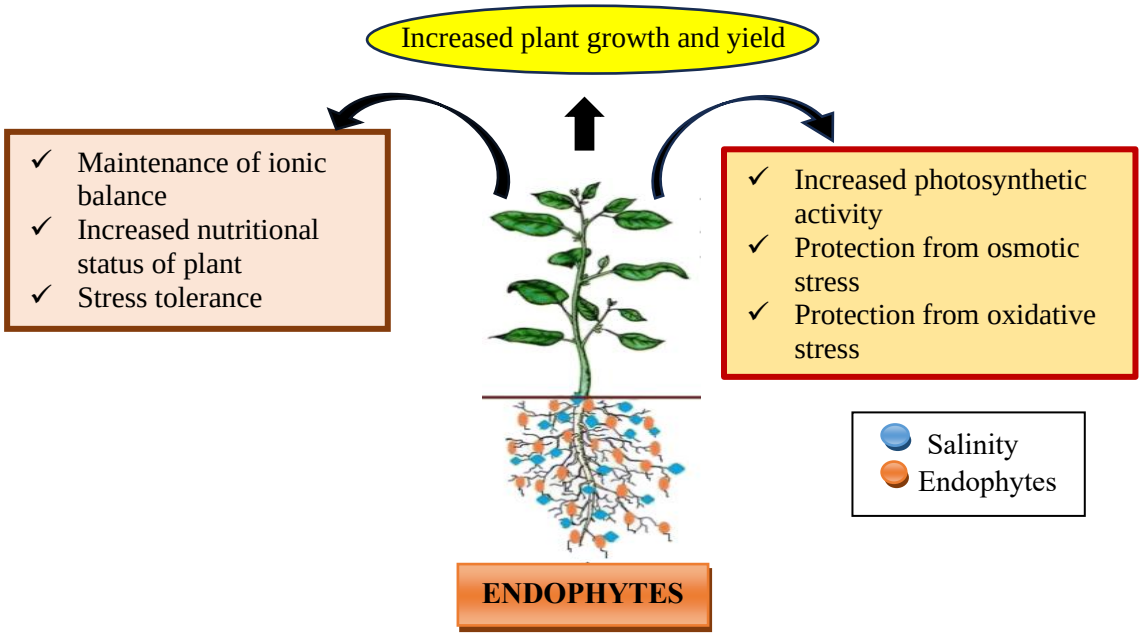

Fig. 2: Effect of Endophytes on plants under salinity stress.

Endophytes are beneficial microbes that help in various aspects of plant growth and development such as nitrogen fixation, siderophore production, phosphate, potassium, and zinc solubilization and can modulate the levels of phytohormones like auxin, cytokinins, and gibberellins within the plant and facilitate the acquisition of some nutrients such as nitrogen, iron and phosphorus by solubilizing them from a complex form and can also produce vitamins (Santoyo et al., 2016; Lambert and Joos, 1989).

Endophytes can be extracted from the inner parts of the plant or isolated from surface-sterilized plant tissues (Hallmann et al., 1997). Endophytic microorganism has been widely reported from roots, stems, leaves, needles, seeds, barks, and twigs of various plant species and the role of the endophytic microbial community in endophyte plant associations have been intensively discussed (Seena and Sridhar, 2004; Reinhold-Hurek and Hurek, 2011). The non-pathogenic endophytic bacteria can increase the plant's resistance to biotic and abiotic stresses (Dimkpa et al., 2009). The role of endophytic microorganisms

in the advancement of plant growth has received growing attention in recent years with the introduction or manipulation of endophytic microorganisms may provide a consistent and effective enhancement in the productivity of major crops (Morris [illegible] al., 2010).

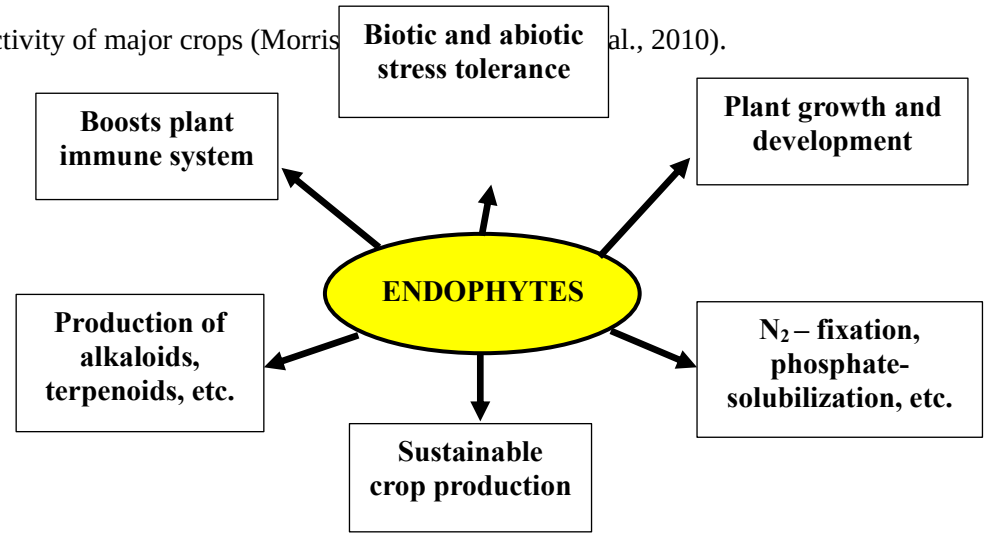

Fig. 3: Various roles of endophytes.

Endophytes are one of the inexpensive, environmentally friendly solutions that may be used to control biotic and abiotic stress conditions and boost crop output. Plants are inoculated with beneficial bacteria, which stimulate plant growth and increases salt tolerance. By causing physical and chemical changes in plant cells that leads to an increased tolerance to environmental stress, is commonly known as "induced systemic tolerance," they are able to adapt to harsh environmental conditions and protect plants from the harmful effects of environmental stresses (Yang et al., 2009).

REVIEW OF LITERATURE

Systematic position of the plant

According to system of classification of Arthur Cronquist (1981), *Cicer arietinum* occupies the following systematic position:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Cicer*

Species: *arietinum* L.

Description of the plant

Chickpea plant grows quickly, branched and attains a height of 20 to 60 cm or even 1 m. It contains multiple secondary lateral roots that delve into the top soil layers in addition to a large taproot that can reach a depth of two meters (15–30 cm). The leaves are 5 cm long and have ten to twenty sessile, oblong to elliptic leaflets. The stems are hairy, straight or twisted, simple or branching. The single, papilionaceous flowers are pink, white, blue, or purple in color (Benkadri et al., 2018; Kushwaha et al., 2021).

On an average, single plant produces 50 to 60 pods which are pubescent, inflated, and oblong. The seeds (usually one seed per pod) are in the range of different colours, shapes, and sizes (creamy-white to black; spherical to angular; 5 to 10 mm in diameter). In India, it is usually grown in a cool season rainfed in semi-arid regions, or as a dry climate crop (Koul et al., 2022)

Bacterial endophytes isolated from different plants and their role in plant metabolism:

It is well known that the endophytic population varies from plant to plant and from species to species. The same plant species may exhibit various endophytic microorganisms growing in various locations. As a result, changes in the climate have an impact on the growth of endophytes **(Nair & Padmavathy, 2014)**. In literature various studies are available related to isolation of endophytes from various parts of different plants and their functions in plant metabolism i.e., growth supporting, tolerance to their host plants against herbivory, salinity, drought, and pathogen stress but the mechanism by which this is done has not yet been revealed in detail **(Redman et al., 2011; Hamilton and Bauerle, 2012; Zhou et al., 2015; Azad and Kaminskyj, 2016; Cosme et al., 2016)**. Some recent literature is cited here in support of different endophytes isolated from different plants and their role in plant metabolism

Syed et al., (2023) isolated cultures of *Pseudomonas fluorescence* and *Trichoderma* spp. from heavy metal-contaminated soil and found that lowering Cd uptake increased chickpea growth and yield.

According to **Di et al., (2023)**, priming of sugarcane sets with endophytic strain *Bacillus subtilis* B9 dramatically accelerated the growth of plantlets and raised the levels of macronutrients leads to enhanced photosynthetic rate, root growth, and chlorophyll content over the control. This study suggested that use of endophytic strain *Bacillus subtilis* B9 as a biofertilizer for sugarcane crop can reduce chemical fertilizer application.

Jhuma et al., (2021) isolated 75 endophytic bacteria from the root samples of *Oryza sativa.* Four of which *Enterobacter cloacae, Achromobacter xylosoxidans, Bacillus aryabhattai,* and *Stenotrophomonas pavanii* identified as salt tolerant endophytic bacteria.

Sampangi-Ramaiah et al., (2020) isolated Endophytes from leaf, root, and seeds from the salt-sensitive IR-64 variety and salt-tolerant Pokkali rice varieties. They isolated total 494 endophytic fungi out of which 20.65% from the shoots, 29.95% from the roots and 49.40% derived from the seeds. They isolated endophytic fungi such as *Gibberella intermedia* and *Fusarium solani*. These endophytic fungi grow on high salt concentration even on 1.5 M and 2 M NaCl concentration. They successfully transfered the salt-tolerant endophytes, from the salt adapted rice variety, Pokkali, to a salt sensitive rice variety, IR-64, making the latter salt-tolerant.

Brigido et al., (2019) aimed to isolate, identify and characterize culturable endophytic bacteria from chickpea (*Cicer arietinum* L.) roots grown in different soils and revealed that the endophytic bacteria isolated in this work belong to the phyla Proteobacteria, Firmicutes and Actinobacteria, with *Enterobacter* and *Pseudomonas* being the most frequently observed genera. Production of indoleacetic acid and ammonia were the most widespread plant growth-promoting features.

Abd-Alla et al., (2019) isolated *Rhizobium sp*. (MK358859) from the root nodules of chickpea. The impact of seven soil salinity levels (0, 25, 50, 75, 100, 150, and 200 mM NaCl) on germination and subsequent growth was measured. The salinity levels ranging from 25 to 150 mM significantly inhibited the growth of chickpea plants, while the 200 mM level hindered their germination. In this study they concluded that triple microbial inoculation (mycorrhizal fungi, Fe_3O_4 NP-induced *Rhizobium*, and endophytic *Stenotrophomonas maltophilia)* could be a successful bio-fertilizer that can contribute to protecting chickpea plants from salinity by attenuating salt-induced oxidative damage.

Walitang et al., (2018) isolated endophytic bacteria from five salt-tolerant rice cultivars IC27, IC31, IC32 and IC37. The six isolates, belonging to *Flavobacterium, Pantoea, Enterobacter, Microbacterium, Kosakonia* and *Curtobacterium* groups identified as salt tolerant endophytic bacteria. These endophytic bacterial strains reported to induce salt tolerance to salt-sensitive rice cultivar (IR29).

El-Awady et al., (2015) isolated 19 endophytic bacteria from roots and stem of *Sesuvium verrucosum* grown in extreme saline conditions. The isolated strains of Endophytic bacteria showed different level

of tolerance to the increasing salt concentration. The six isolates were identified as salt-tolerant endophytes: *Enterobacter cancerogenus*, *Vibrio cholerae, Bacillus subtilis*, *Escherichia coli* and two *Enterobacter* sp. which grow well under all NaCl concentrations and tolerate 7.5 % and 15% NaCl concentration.

Damodaran et al., (2013) isolated 16 endophytic bacteria from sodic rhizospheres of roots of Phyto-ameliorant grasses, five of them showed tolerance to high salt concentration (7.5 % NaCl) and isolated *Bacillus pumilus* and *B. subtilis* had higher sodium uptake when cultured under in-vitro conditions in 1 M NaCl solution. These bacteria are more likely to withstand saline conditions.

Asis et al., (2000) had confirmed the presence of N_2-fixing endophytic bacteria in the Japanese sugarcane cv. NiF-8.

Some other fungal endophytes isolated from different plants are listed below

Table 1: Some other fungal endophytes isolated from different plants

Endophytes	Plant species	References
Phomopsis sp.	*Neolitsea sericea* *Pasania edulis* *Ginkgo biloba* L. *Tectona grandis* and *Samanea saman* Merr. *Taxus chinensis*	Hata and Sone, 2008 Hata et al., 2002 Thongsandee et al., 2012 Chareprasert et al., 2006 Liu et al., 2009
***Cladosporium* sp.**	*Opuntia ficus indica* *Cinnamomum camphora*	Bezerra et al., 2012 He et al., 2012
Cladosporium herbarum	*Lycopersicum esculentum Mill.* *Triticum aestivum*	Larran et al., 2001 Larran et al., 2002
***Colletotrichum* sp.**	*Triticum aestivum* *Citrus* plants *Cinnamomum camphora* *Pasania edulis* *Ginkgo biloba* L. *Tectona grandis* and *Samanea saman* Merr. *Huperzia serrata* *Cinnamomum camphora*	Larran et al., 2002 Glienke-Blanco et al., 2002 He et al., 2012 Hata et al., 2002 Thongsandee et al., 2012 Chareprasert et al., 2006 Wang et al., 2011 He et al., 2012
Colletotrichum gloeosporiodes	*Lycopersicum esculentum*	Larran et al., 2001
***Phyllosticta* sp.**	*Citrus sp.* *Pasania edulis* *Coffea arabica* *Quercus variabilis* *Centella asiatica* *Panax quinquefolium* *Ginkgo biloba* L.	Glienke-Blanco et al., 2002 Hata et al., 2002 Santamaria and Bayman, 2005 Wang et al., 2007 Rakotoniriana et al., 2008 Xing et al., 2010 Thongsandee et al., 2012

***Penicillium* sp.**	*Lycopersicum esculentum* Mill. *Huperzia serrata*	Larran et al., 2001 Wang et., 2011
***Acremonium* sp.**	*Taxus chinensis* *Huperzia serrata*	Liu et al., 2009 Glienke-Blanco et al., 2002

OBJECTIVES OF THE STUDY

Taking the aforementioned views in due consideration, the present study is proposed to be undertaken with the following objectives:

1. *In-vitro* screening of endophytes to evaluate their growth promoting activity.

2. To study the impact of seed priming with selected endophytes on seed germination and seedling attributes of *Cicer arietinum* L. under salinity stress.

3. To study the effect of soil drenching with selected endophytes on the growth, physio-chemical and yield parameters of *Cicer arietinum* L. under salinity stress.

4. Understanding the impact of combined application of seed priming and soil drenching on growth, physio-chemical and yield parameters of *Cicer arietinum* L. under salinity stress.

MATERIALS AND METHODS

1. ***In-vitro* screening of endophytes to evaluate their growth promoting activity**

The endophytes will be screened and selected on the basis of best performance in respect of following plant growth promoting traits:

- Ammonia production : For screening of the production of ammonia by endophytes, the protocol of Cappuccino and Sherman, 1992 will be followed.
- Salinity tolerance : NaCl will be added into nutrient agar medium at various concentrations and the test bacterial endophytes will be streaked.
- Phosphate-solubilization : For phosphate solubilization, the protocol of Jasim *et al*., 2013 using Pikovskaya medium and bromophenol blue as indicator will be followed.
- Potassium solubilization : For potassium solubilization, the protocol of Sugumaran and Janartham, 2007 using Aleksandrov agar medium will be followed.
- IAA production : In order to screen for the endophytes producing IAA, a colorimetric assay will be performed following the protocol of Ehmann, 1977.
- Siderophore production: Productions of siderophores by bacterial cultures will be detected on the

Chrome-azuorol S medium (CAS medium) as described by Schwyn and Neilands, 1987.

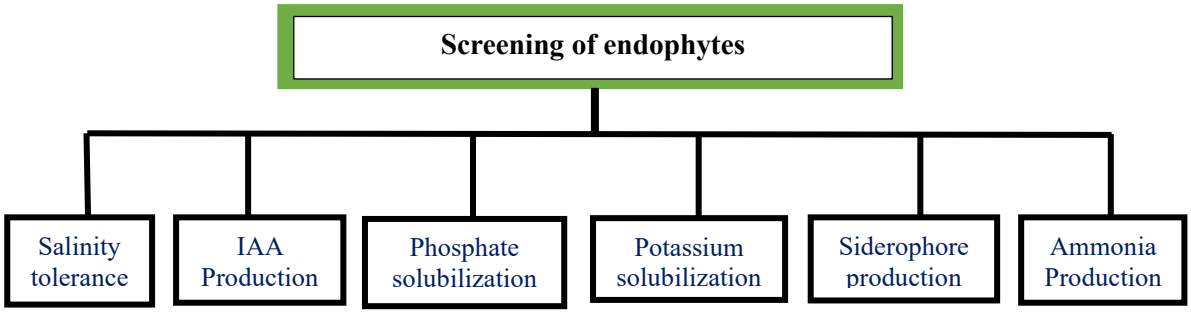

Fig. 4: Parameters on which the endophytes will be screened and selected.

2. **To study the impact of seed priming with selected endophytes on seed germination and seedling attributes of chickpea (*Cicer arietinum* L.) under salinity stress**

Cicer arietinum L. seeds will be collected from authentic source.

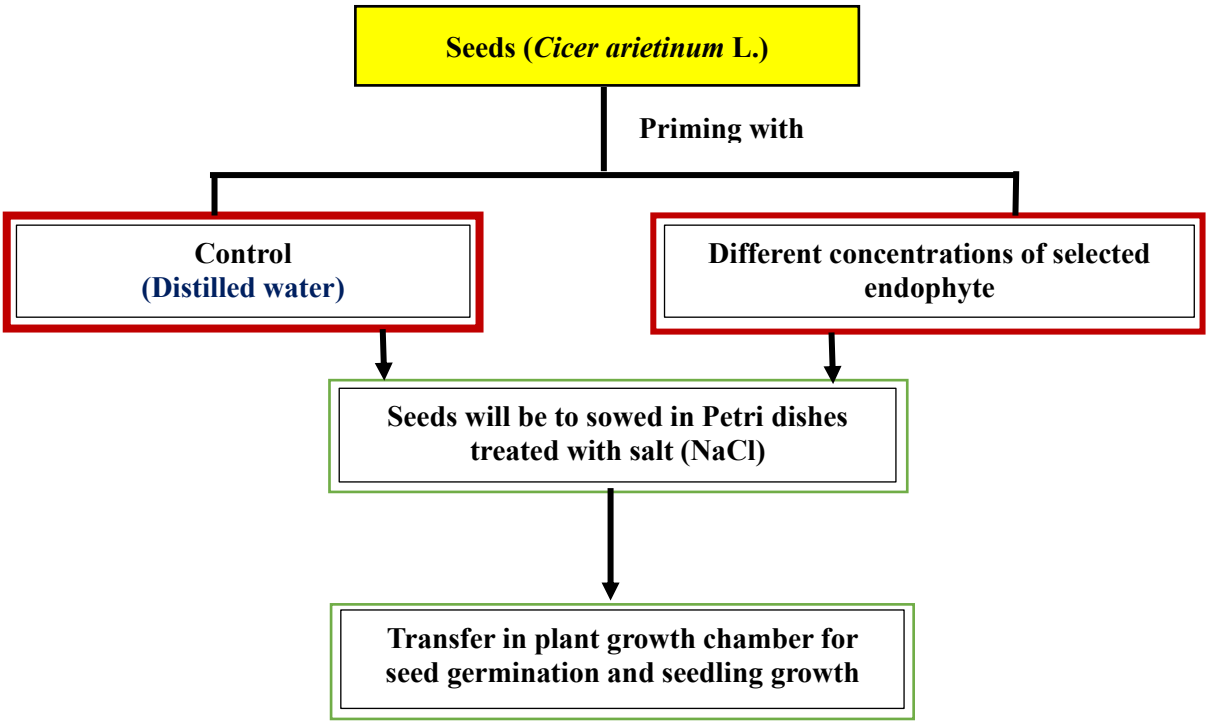
Seeds (Cicer arietinum L.)
Priming with
Control (Distilled water)
Different concentrations of selected endophyte
Seeds will be to sowed in Petri dishes treated with salt (NaCl)
Transfer in plant growth chamber for seed germination and seedling growth

Fig. 5: Seed priming of the seeds with the selected endophyte

After germination, the following parameters will be determined:

i. Germination potential (GP)

ii. Germination rate (GR)

iii. Mean germination time (GT)

iv. Germination index (GI)

v. Seed vigour index

Data will be recorded at regular intervals of 3 days of sowing (DAS).

3. **To study the effect of soil drenching with selected endophytes on the growth, physio-chemical and yield parameters of chickpea (*Cicer arietinum* L.) under salinity stress**

For the soil drenching, the different treatments of the selected endophytes suspensions will be applied in equal dose around the root zone of each seedling. In the control, sterile distilled water will be applied in the same way.

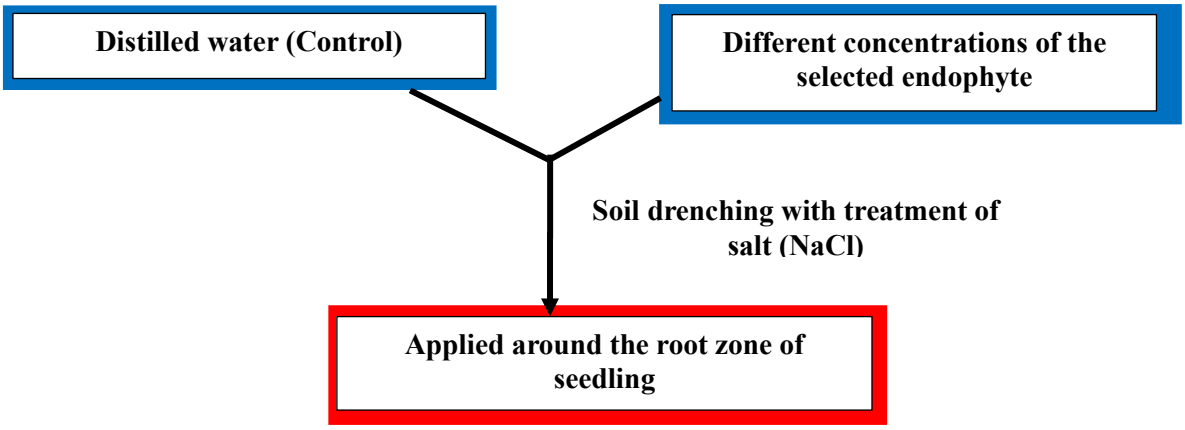
Distilled water (Control)
Different concentrations of the selected endophyte
Soil drenching with treatment of salt (NaCl)
Applied around the root zone of seedling

Fig. 6: Soil drenching with the selected endophyte

5. **To study the combined effect of seed priming and soil drenching with selected endophytes on growth, physio-chemical and yield parameters of chickpea (*Cicer arietinum* L.) under salinity stress.**

Firstly, the seed priming will be done. After the germination of the seedlings, they will be transferred in the pots filled with soil then soil drenching will be done in which the different concentrations of the selected endophyte suspension will be applied around the root zone of the seedling along with the treatment of salt (NaCl) to create salinity stress.

After completion of all treatments, the estimation of the following will be done:

A) Growth parameters:

i. Root length (cm)

ii. Shoot length (cm)

iii. Number of branches per plant

iv. Leaf area (cm^2)

v. Root fresh weight (gm)

vi. Shoot fresh weight (gm)

vii. Shoot dry weight (gm)

viii. Root dry weight (gm)

B) **Physiological and biochemical parameters:**

i. Total chlorophyll content (Lichtenthaler et al., 2001).

ii. Total carotenoid content (Lichtenthaler et al., 2001)

iii. Photosynthetic rate by IRGA (Infrared Gas Analyzer)

iv. Stomatal conductance by IRGA (Infrared Gas Analyzer)

v. Electrolyte leakage by EC meter

vi. Leaf-nitrogen content by Kjeldahl method

vii. Leaf-phosphorus content (by Spectrophotometer)

viii. Leaf-potassium content (by Flame photometer)

ix. Lipid peroxidation (Heath and Packer, 1968)

x. Hydrogen peroxide (H_2O_2) (Velikova et al., 2000)

xi. Proline content (Bates et al., 1973)

xii. Antioxidant assay

- CAT (catalase EC1.11.1.6) (Aebi, 1984).
- POD (peroxidase EC1.11.1.7) (Zhang, 1992).
- SOD (Superoxide dismustase EC1.15.1.1) (Giannopolitis and Ries, 1977).

xiii. Relative water content (RWC) (Kaya et al., 2013).

xiv. Seed carbohydrate content (Sadasivam, 1962)

C) **Yield parameters:**

i. Pod bearing branches

ii. Pods per plant

iii. Number of seeds per pod

iv. 100 seed weight

6. Statistical analysis

Statistical analysis of recorded data will be done by statistical software.

IMPORTANCE OF STUDY

- In future, salinity is expected to increase, which will inevitably increase the severity of abiotic stresses. Present study could be an **Eco-friendly approach to enhance chickpea yield** under salinity stress condition.
- The use of endophytes in agricultural practises may **contribute to the preservation of the ecological stability** of agro-ecosystems.
- The use of endophytes may offer a **long-term and affordable solution** to plant productivity losses brought on by shifting climatic conditions.
- **New approaches to the construction of sustainable agriculture** so that excessive fertilizer and pesticide use as productivity boosters can be avoided.
- **Incorporating environmentally friendly practices into agriculture** that can offer suitable answers for raising productivity is in high demand on a global scale.

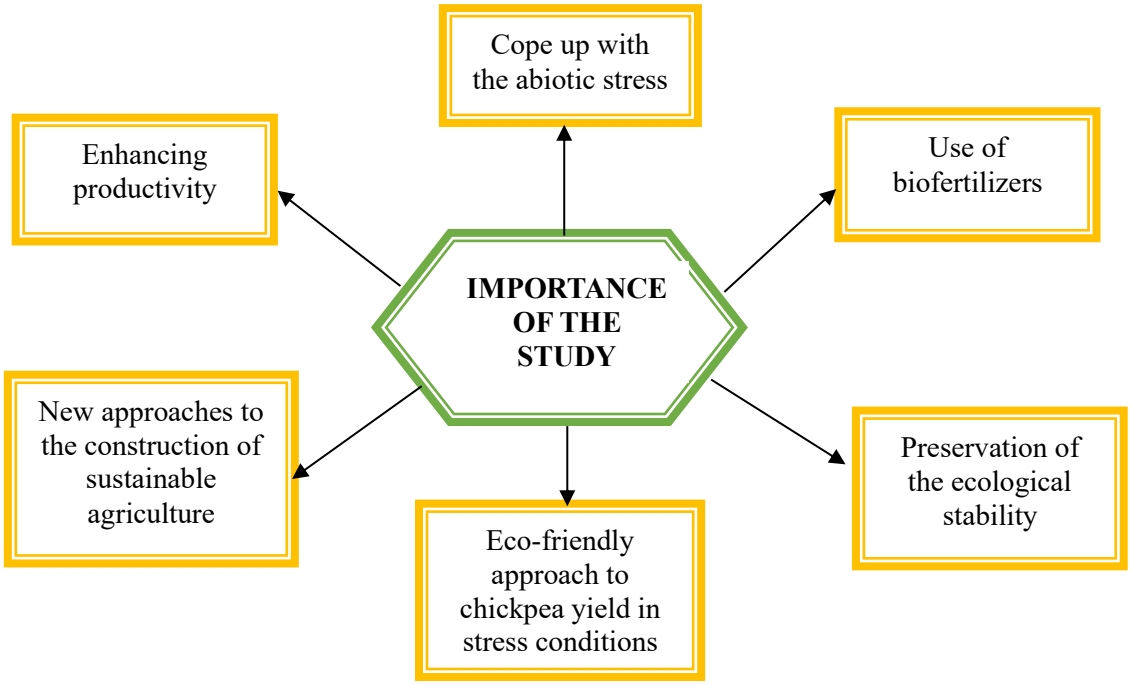
Cope up with the abiotic stress
Enhancing productivity
Use of biofertilizers
IMPORTANCE OF THE STUDY
New approaches to the construction of sustainable agriculture
Preservation of the ecological stability
Eco-friendly approach to chickpea yield in stress conditions

Fig. 7: Various importance of the study.

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